Modulation of Gene Expression Induced in Human Epidermis by Environmental Stress *In Vivo*

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Environmental insults on the skin induce biologic responses through the modulation of expression of genes implicated in different cell functions. The aim of this study was to investigate the modulation of gene expression profile in human epidermis *in vivo* following different stresses. We determined the modulations of gene expression using cDNA macroarray in the epidermis of 28 healthy volunteers, following mild and physiologic insults, including: (1), tape stripping; (2) application of 10% sodium dodecyl sulfate; (3) daily application of vaseline; and (4), exposure to one minimal erythema dose of solar-simulated radiation. The analysis was performed 19 h after treatment. The reverse transcription–polymerase chain reaction method was used to confirm our results. We showed that: (1) the intensity of gene modulation was variable among the volunteers following the same skin stress; (2) the nature and intensity of skin treatment modified the pattern of gene expression; and (3) some genes were modulated only by specific stress, some others are modulated irrespective of the stress. GADD45, Bax, SAS, and granulocyte chemotactic protein-2 were overexpressed exclusively following solar-simulated radiation, whereas tape stripping led to the modulation of genes implicated in different pathways (inflammation, cell proliferation, cell differentiation, detoxification, etc.). Concerning common gene modulation, MRP8 and MRP14 were highly upregulated in human skin epidermis after solar-simulated radiation, vaseline application or tape stripping, and to a lower extent after sodium dodecyl sulfate. Such upregulation of the MRP 8/14 genes was confirmed at the protein level in an *ex-vivo* skin culture model following tape stripping and solar-simulated radiation. Together, these results suggest that MRP8 and MRP14 may be general, yet highly sensitive, markers for a great variety of skin stresses and that they are implicated in several epidermal repair pathways. ***Key words: cDNA array/epidermis/gene profiling/sodium dodecyl sulfate/tape stripping/ultraviolet radiation/vaseline. J Invest Dermatol 121:1447–1458, 2003***

An important function of skin is to act as a barrier and thus provide protection against a variety of environmental stresses such as mechanical, chemical, or solar ultraviolet stress. Soon after these stresses a cascade of molecular events is induced aiming at restoring normal skin homeostasis.

Disruption of the epidermal permeability barrier assured by the extracellular lipids of the stratum corneum (SC) within which the corneocytes are embedded, is associated with loss of corneocyte cohesiveness, desquamation, increasing transepidermal water loss (TEWL), and subsequent dehydration of the skin. Acute disruption of the permeability barrier may be achieved by various experimental treatments, including the application of organic solvents such as acetone, detergents, e.g., sodium dodecyl sulfate (SDS) or by repeated tape stripping, a physical insult that removes SC layers. A homeostatic repair response is initiated whose aim is to restore normal epidermal barrier function. At cellular level, the repair response includes increased keratinocyte proliferation in the basal layer (Proksch *et al*, 1993). At the biochemical level, repair responses include: (1) rapid secretion of preformed lamellar bodies by the stratum granulosum cells; (2) accelerated formation of new lamellar bodies; and (3), increased synthesis of extracellular lipids of the SC (Menon *et al*, 1985, 1992; Grubauer *et al*, 1987; Proksch *et al*, 1990; Holleran *et al*, 1991). In addition, disruption of the cutaneous permeability barrier can lead to molecular responses including a rapid increase in DNA synthesis and early overexpression of several genes such as genes coding for cytokines (Wood *et al*, 1992, 1997; Nickoloff and Naidu, 1994), genes implicated in keratinocyte growth and differentiation (Healy *et al*, 1994, 1995; Le *et al*, 1996; Liou *et al*, 1997), and genes coding for key enzymes of SC lipid synthesis and transport (Harris *et al*, 1997, 1998). The link of cytokines to barrier homeostasis remains unclear because occlusion does not abrogate cytokine production in the context of acute barrier disturbances (Wood *et al*, 1994). The increase in the expression of genes implicated in keratinocyte growth and differentiation may be responsible for the increased proliferative response that follows barrier disruption.

Solar UV light that reaches the earth surface is composed of UVB (290–320 nm) and UVA (320–400 nm), which differ in

Manuscript received February 27, 2003; revised April 24, 2003; accepted for publication June 25, 2003

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Abbreviations: MED, minimal erythema dose; SC, stratum corneum; SSR, solar-simulated radiation; TEWL, transepidermal water loss; ultraviolet, UV.

0022-202X/03/$15.00 ·

their biologic effects. It has been shown that UVB can alter barrier function (Haratake *et al*, 1997) but the major effect of UVB on skin is characterized clinically by erythema, and histologically by the formation in the epidermis of sunburn cells, which are considered to be apoptotic keratinocytes (Young, 1987; Maytin *et al*, 1994; Bernerd *et al*, 1999). At a molecular level, UVB radiation is absorbed by macromolecules, including DNA and proteins. It was shown to be a strong DNA-damaging agent (Wikonkal and Brash, 1999). In contrast UVA radiation is weakly absorbed by most biomolecules, but generates active oxygen species via a variety of chromophores (Tyrrell, 1996). Data concerning the activation of gene expression following UV light irradiation are often restricted to *in vitro* models. They include the UVB induction of oncogenes (e.g., *c-fos* and *c-jun*) or the p53 tumor suppressor gene, as well as cytokines such as interleukin (IL)-1, tumor necrosis factor-α, or IL-10 (see review in Tyrrell, 1996). Recently, analysis of UVB response in human keratinocytes using cDNA microarrays showed the modulation of genes implicated in DNA repair, inflammation, apoptosis, and cell adhesion (Becker *et al*, 2001; Li *et al*, 2001; Sesto *et al*, 2002).

Until recently, differential gene expression was usually analyzed by a step-by-step approach, using techniques such as reverse transcriptase polymerase chain reaction (PCR) or northern blot, looking for genes preselected a priori. These methodologies are not suitable for global and simultaneous analysis of complex changes of gene expression pattern that characterize skin response to environmental injury. During the last 5 y, the rise of DNA macroarray and microarray technologies has made it possible to analyze the regulation of gene expression without a priori in a highly parallel and rapidly serialized manner.

The aim of this study was to investigate the alteration of gene expression profiles in human epidermis *in vivo* following various types of environmental stress in a large and physiologic manner. For this purpose, the modulation of gene expression was determined using a cDNA macroarray technology in the epidermis of several healthy volunteers following mild and physiologic stress. The stress models were chosen to be representative of several types of real life situations. They included: (1) tape stripping (removing all or part of the SC, mimicking mechanical injury); (2) application of 10% SDS detergent (mimicking the effect of an aggressive soap); (3) daily application of vaseline (considered as a "rustic" cosmetic); and (4), exposure to a physiologic dose (1 minimal erythema dose (MED)) of solar-simulated radiation (SSR; reproducing sun exposure). In all conditions, the analysis was performed 19 h after treatment, in order to focus on late modifications of gene expression.

MATERIALS AND METHODS

Volunteers All volunteers were Caucasians, with phototypes II or III, with no past or present history of allergy or any skin disease. They all gave informed consent and the bioethics committees of Saint-Louis and Boucicaut Hospitals, Paris, France approved the study. The distribution of volunteers in the different protocols was as follows.

Tape-stripping Ten healthy male volunteers (mean age of 27 y, range 21–33 y), coded A to J.

SDS Six healthy male volunteers (mean age 26 y, range 22–32 y).

Vaseline Six healthy male volunteers (mean age of 28 y, range 23–32 y), coded K to P.

SSR Six healthy, nonpregnant, female volunteers (mean age of 24 y, range 21–26 y), coded Q to V.

Treatments For tape stripping, SDS and SSR treatments, the exposed skin area was 2.5 × 2.5 cm; for vaseline, it was 2.5 × 5 cm.

Tape stripping Sellotape stripping of the skin was carried out on two areas on one inner forearm. Individual pieces of sellotape (Blenderm, 3M) were applied to and peeled from one area of the skin in a sequential manner until the skin glistened. The clinical aspect of skin following this treatment was a definite sign of skin denudation corresponding to a complete removal of the SC (Pinkus, 1951). This treatment was referred to as "strong tape stripping" with a mean of 40 strippings ± 1 (mean ± SEM, n = 10). On another area of the forearm, half the number of strippings was carried out. This treatment was referred to as "moderate tape stripping" with a mean of 20 strippings ± 0.6 (mean ± SEM, n = 10). The skin of the other inner forearm served as a control. The three areas were then covered with dry gauze.

SDS Volunteers were painted on an area of one inner forearm with 10% SDS as follows: 10 gentle applications were performed four times successively with a 10% SDS-soaked cotton ball. At the end of each 10 application sequence the skin was rinsed with distilled water. The other inner forearm was not treated. Both experimental areas were then covered with dry gauze.

Vaseline Volunteers had to massage the skin for 10 s with 2 mg per cm^2 of vaseline on a defined area of one inner forearm, twice a day (morning and evening) for 7 d. The other inner forearm had to be massaged the same way but without vaseline and served as a control.

SSR SSR radiation source was an Oriel 1000 W xenon arc solar simulator equipped with a Schott WG320 filter and a dichroic mirror. The spectral irradiance distribution at skin level (40 cm from the lamp) was measured with a calibrated spectroradiometer 3010 (Macam, Livingston, Scotland, UK) (**Fig 1**). The output was monitored with a RMX3W radiometer (Vilber-Lourmat, Marre la Vallee, France) equipped with UVA and UVB sensors. The untanned buttock region was used as the exposure site. For each individual, the MED was determined by chromametry using a CR200 chromameter (Minolta, Tokyo, Japan) before exposure on the same part of the body (MED erythema standard threshold is defined as Delta $a^* = +2.5$). The average individual MED was approximately 2.32 J per cm^2 (UVA + UVB). Thereafter, two areas were randomized and delineated on the buttock of volunteers. One area was exposed to 1 MED, whereas the other one served as an unexposed control. Nineteen hours after exposure, chromametric assessment of the erythema was performed.

TEWL measurements For tape stripping, SDS, and vaseline treatments, TEWL was measured with an EP1 evaporimeter (Servomed, Cairo, Egypt) on each of the experimental areas of both inner forearms. Measurements were performed before, immediately after, and 19 h after tape stripping and SDS treatments. For vaseline treatment, TEWL was measured 19 h after the last application.

Tissue collection For each protocol, epidermis samples of the experimental areas were removed under local anesthesia, using a dermatome GA630 (AESCULAP, Melsungen, Germany). Preliminary trials to determine the appropriate sample thickness were performed using surgical material from abdominal skin flaps. Five micrometer cryostat sections were stained with 0.5% toluidine blue, 1% borax. These experiments indicated that 0.2 mm was the optimal thickness that allowed harvesting the

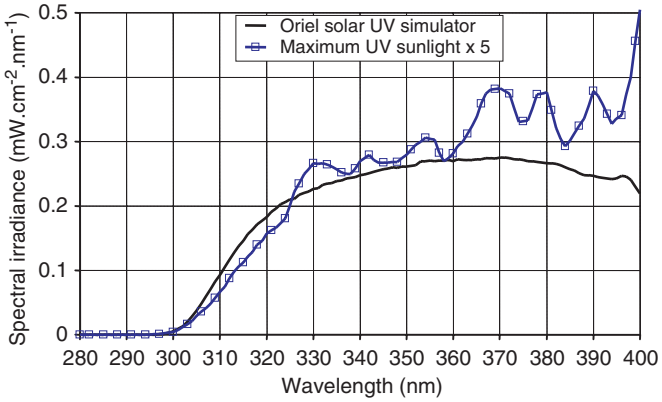

Figure 1. Spectral irradiance of solar UV simulator and maximum sunlight. Maximum sunlight corresponds to a representative summer noontime solar spectrum measurement from Albuquerque, New Mexico (Sayre *et al*, 1990). The solar UV simulator irradiance was measured with a spectroradiometer Bentham DM150 (Reading, UK) calibrated using lamps traceable to the National Physics Laboratory (Teddington, UK).

epidermis as a whole, including villosities, with minimal dermal contamination (**Fig 2**). The sample area was 1.5 × 1.5 cm.

RNA extraction Epidermis samples were disrupted in lysis buffer (Rneasy mini-kit, Qiagen) immediately after collection, using a glass potter. Tissue lysates were homogenized using QIAsredder columns (Qiagen Hiden, Germany) and total RNA was obtained according to the manufacturer's instructions using Rneasy mini-kit (Qiagen). Dnase I treatment (27 units, 15 min) of total RNA was performed directly on the spin columns to eliminate genomic contamination of the RNA samples. Concentration and purity of total RNA were determined by measuring the absorbance at 260 nm and 280 nm. The average yield of total RNA extraction was 17 ± 1 μg per cm^2 (mean ± SEM, n = 74). Agarose gel electrophoresis and ethidium bromide staining checked integrity of RNA preparations. Purified, Dnase I-digested, RNA samples were aliquoted and snap-frozen, then kept at –80°C until use.

Differential hybridization of Atlas Human cDNA expression arrays (588 named genes, Clontech no. 7740-1) Microarray analyses were performed following the manufacturer's instructions. Briefly, 5 μg of total RNA were converted to ^{33}P-labeled cDNA using [α-^{33}P]deoxyadenosine triphosphate (Amersham Piscataway, NJ), Powerscript reverse transcriptase, and a set of "Atlas Human cDNA synthesis" specific primers (Clontech Palo Alto, CA). Equal amounts of radiolabeled cDNA probes from control and treated epidermis of each volunteer were used for parallel hybridizations of Atlas arrays from the same production batch. Following overnight hybridization at 68°C, array membranes were washed four times in 2 × sodium citrate/chloride/1% SDS buffer for 30 min at 68°C, followed by two washes in 0.1 × sodium citrate/chloride/0.5% SDS buffer for 30 min at 68°C, and exposed to a Kodak Phosphorimager screen for 5 d. Hybridization signals were acquired with a Storm 840 phosphorimager.

Image analysis and data collection The Software program Imagene version 4.1 (Biodiscovery El Secundo, CA) was used to quantify and normalize the data. For each volunteer, data from the control array were compared with data from the treatment array(s). Spots with low signals in both membranes (difference between signal spot and background = 1.0) were not considered for further analysis. Local background signal was subtracted from the intensity of each spot. For each volunteer, the level of modulation was determined as the ratio of the intensity of each spot from the treated sample divided by the signal of each spot from the control sample. Ratios were normalized with all spots of the filters having ratios lower than 2 SD of all spot ratios (iterative algorithm).

Statistical analysis Modulated genes were selected as follows: genes with at least 2-fold modulation in at least 50% of the volunteers (five of 10 or three of six) were selected, the other volunteers showing ratios comprised between 0.5 and 2.0. A Student's t test was performed on the log of the ratio to calculate whether the geometric mean ratio of these genes was statistically different from 1.0 ($p < 0.1$).

TEWL means were compared using nonparametric two-tailed Wilcoxon test.

Semiquantitative reverse transcription–PCR. One microgram of total RNA was used for first strand cDNA synthesis using an Advantage RT-for-PCR kit (Clontech) according to the manufacturer's instructions; 1/20th of the synthesized cDNA was used as a template for PCR using a Titanium Taq PCR kit (Clontech) and gene-specific primer sets listed in **Table I**. After 1 min of heat denaturation at 94°C, each cDNA of interest was amplified by the following iterative incubations: 30 s at 94°C, 2 min at 68°C, and a final, unique step at 68°C for 5 min. Samples corresponding to 1/10th of each reaction were taken after 20, 25, 30, and 35 PCR cycles, separated on 2% agarose gel, followed by ethidium bromide staining. Signals were quantified with a Biorad Imager Gel Doc densitometer (Biorad, Hercules, CA) and normalized against a housekeeping gene.

Histology of human skin: skin sample and treatments Human skin (phototype II, 36 y old) obtained from plastic mammary reduction was used to study localization of MRP14 and MRP8 proteins following treatments. Tape stripping corresponded to 40 strippings and SSR exposure was performed using a dose equivalent to 1 MED, i.e., leading to the induction of three sunburn cells per mm of epidermis in mean (Sheehan and Young, 2000). Following treatment, pieces of human skin were maintained *ex vivo* in air-exposed conditions during 24 h as described (Bernerd and Asselineau, 1997). Half of each sample was frozen in liquid nitrogen for immunostaining, the other half was fixed in 10% formalin for classical histology (hematoxylin, eosin, saffron).

Immunostaining of human skin

Antibodies Mouse monoclonal antibodies against human MRP8 (8-5C2; BMA Biomedicals Switzerland) were used diluted 1/25, and MRP14 (S36.48; BMA Biomedicals Angst Switzerland), used diluted 1/50. Fluorescein isothiocyanate-conjugate rabbit anti-mouse immunoglobulins (Dako, Glosstrup, Denmark) was used diluted 1/80 as the second antibody.

Procedure Frozen samples were embedded in Tissue-Tek (Sakura, Zoeterwerde, the Netherlands). Vertical cryosections (5 μm) were prepared for immunohistochemistry. Immunolabeling MRP14 and MRP8 were performed on air-dried sections, rinsed in phosphate-buffered saline (Biomerieux Laboratories, Marcy l'etoile, France), and incubated at room temperature for 1 h with the first antibody, rinsed with phosphate-buffered saline, incubated with the second conjugated antibody for 30 min, washed, and mounted in 90% glycerol in phosphate-buffered saline containing 5 mM p-phenylenediamine, before being observed under a fluorescence microscope.

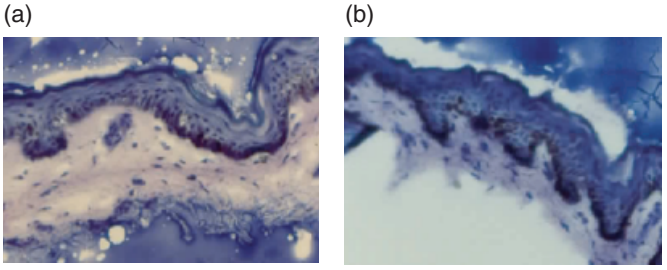

Figure 2. Histology of skin after dermatome cutting. The thickness of skin cutting was adjusted on dermatome to 0.2 mm (*a*) or to 0.3 mm (*b*). Note that the 0.2 mm thickness allowed collection of the entire epidermis with minimal dermal contamination.

RESULTS

Effect of mechanical and chemical treatments on barrier disruption Repeated tape stripping of skin produced an

Table I. Primer sequences used for semiquantitative reverse transcription–PCR

Name	Forward sequence	Reverse sequence	Length of amplified fragment
FRA1	5′-CCCTGCCGCCCTGTACCTTGTATC-3′	5′-AGACATTGGCTAGGGTGGCATCTGCA-3′	280 bp
MRP14	5′-GCTCCTCGGCTTTGACAGAGTGCAAG-3′	5′-GCATTTGTGTCCAGGTCCTCCATGATGTGT-3′	238 bp
MRP8	5′-GGGCAAGTTCCGTGGGCATCAGTTG-3′	5′-CCAGTAACTCAGCTACTCTTTGTGGCTTTCT-3′	314 bp
Ribosomal protein S9	5′-GATGAGAAGGACCCACGGCGTCTGTTCG-3′	5′-GAGACAATCCAGCAGCCCAGGAGGGAC-3′	430 bp
gapdh	Amplimer set from Advantage RT PCR kit (Clontech)		983 pb
23 kDa highly basic protein	5′-TAAACAGGTACTGCTGGGCCGGAAGGTG-3′	5′-CACGTTCTTCTCGGCCTGTTTCCGTAGC-3′	483 bp

increase in TEWL (**Fig 3*a***). One minute after strong tape stripping (40 strippings on average), the TEWL reached 65 g per m^2 per h (9-fold induction compared with control area; $p = 0.002$). Barrier disruption was slightly decreased 19 h after strong tape stripping, with a mean value of 54 g per m^2 per h.

Moderate tape stripping (20 strippings on average) led to a 4-fold induction of TEWL compared with control (27.2 g per m^2 per h *vs* 5.7 g per m^2 per h; $p = 0.002$) after 1 min. Nineteen hours after treatment, a 58% barrier recovery was observed.

No significant alteration of epidermal barrier function was detected using TEWL after acute application of 10% SDS (**Fig 3*b***, $p \geq 0.7$) or after daily application of vaseline for 7 d (**Fig 3*c***, $p = 1$).

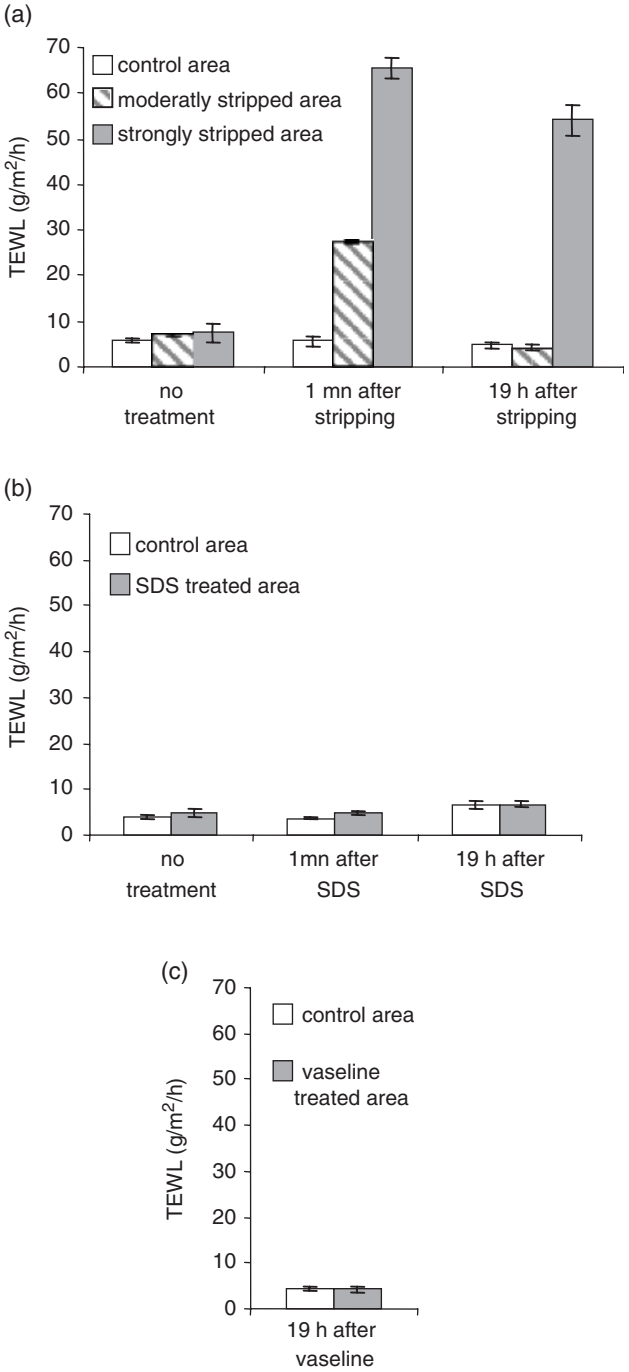

Figure 3. TEWL determination after skin treatment. Changes in TEWL (g per m^2 per h) before and after treatment of the skin with moderate or strong tape stripping (TS; $n = 10$) (*a*), acute application of 10% SDS ($n = 6$) (*b*), daily application of vaseline ($n = 6$) (*c*). Data are expressed as mean ± SEM.

Effect of various skin stresses on human epidermal gene expression The effect of the different skin treatments was studied using differential hybridization of Atlas Human cDNA expression arrays (588 named genes). To identify the genes significantly modulated in each cohort of volunteers, and to eliminate background noise in the analysis of the hybridization experiment we applied stringent filters (see *Materials and Methods*).

The resulting lists of genes modulated following skin treatments are given in **Table II** (strong and moderate tape stripping), III (vaseline), and IV (SSR).

Effect of mechanical stress on human epidermis gene expression Moderate and strong tape strippings were performed on the inner forearm skin of six healthy volunteers. Differential gene expression assays were conducted as described above. Results showed a large gene modulation in the epidermis after strong tape stripping compared with normal epidermis (**Table II**). A total of 37 genes of 588 were modulated following strong tape stripping: 32 genes were overexpressed, whereas five were repressed. These modulated genes belonged to various families of genes. Eleven of 32 (34%) of the overexpressed genes could be classified as genes implicated in extracellular signaling and communication. This set of genes included those that were most strongly activated (mean ratios between 2.3 and 535.4). Both S100 proteins MRP8 and MRP14 had the highest rate of modulation (535.4 and 338.6 on average, respectively). IL-1α, IL-12β, IGF1A, and heparin binding epidermal growth factor-like growth factor also showed strong induction (16–67-fold induction). Several genes coding for proteins that play a part in cellular detoxification and anti-oxidation had their expression altered following strong tape stripping: glutathione S-transferase mu1, glutathione reductase, thioredoxin peroxidase, and cytochrome P450 reductase showed moderate upregulation (2.1–3.5-fold); whereas glutaredoxin was repressed (0.4-fold). Genes encoding transcription factors were either upregulated or downregulated. The protooncogene FRA-1 was induced 63.2-fold over control. The other genes modulated following strong tape stripping were implicated in various cellular functions such as cell adhesion, cell reception, intracellular signaling, cell cycle, apoptosis, stress response, and DNA repair.

Differential gene expression from moderate tape stripping experiments revealed the modulation of seven genes (listed in italics in **Table II**). Six of these seven genes had been found to be strongly modulated after strong tape stripping, i.e., MRP14, MRP8, IGF1A, IGF-BP3, FRA1, and hMLH1. All these genes showed reduced levels of modulation after moderate tape stripping compared with strong tape stripping (e.g., 196-fold *vs* 824-fold induction for MRP14 in volunteer A). One gene appeared to be specifically repressed after moderate tape stripping: *MCAF* (monocyte chemotactic and activating factor). This gene was not selected as modulated after strong tape stripping because of our stringent statistical filter. Specifically, after strong tape stripping one of 10 volunteers in the cohort showed a 3-fold upregulation of this gene, whereas five others showed a repression. Altogether, these results indicated a "dose-effect" of the tape stripping treatment.

Effect of chemical stress on human epidermis gene expression Two genes were upregulated following daily application of vaseline: the MRP14 and MRP8 genes were induced at least 2-fold in five of six volunteers. Volunteer P showed no modulation of those two genes (**Table III**).

Application of 10% SDS on one inner forearm showed no significant modulation of any gene. It is important to note, however, that after 10% SDS, MRP14 and MRP8 expression was induced at least 1.9-fold and 1.7-fold, respectively, in three of six volunteers.

Effect of UV radiation on human epidermis gene expression Following exposure to 1 MED of SSR radiation, differential expression assay revealed upregulation of eight genes (**Table IV**). The class of genes coding for extracellular and communication proteins was most represented, and MRP14 and MRP8 showed the highest ratios of modulation (on average 42-fold induction and 12-fold induction, respectively, compared with control epidermis). The transcription factor FRA1 was induced 10-fold on average compared with control epidermis, with five of six volunteers showing upregulation between 4-fold and 20-fold. Four genes were moderately upregulated following SSR exposure: GADD45, Bax, SAS, and granulocyte chemotactic protein-2 (2–7.4-fold induction). The latter genes were not found to be modulated following tape stripping, vaseline, or SDS treatment. **Table V** summarizes the genes that were found modulated following different conditions of skin treatment. Genes of the S100 family, MRP14 and MRP8, were significantly upregulated in three treatment conditions (tape stripping, SSR, and vaseline) and to a lower extent in the case of 10% SDS treatment (**Fig 4**). FRA1 was induced in human epidermis by tape stripping and by SSR, but we did not detect any modulation of this gene following the chemical treatments (vaseline or 10% SDS).

Reverse transcription–PCR analysis To test the reliability of macroarray hybridization results, the expression of some upregulated genes after different skin treatments was analyzed by semiquantitative reverse transcription–PCR (**Fig 5**). The genes chosen for normalization (genes coding for ribosomal protein S9, 23 kDa highly basic protein, and liver glyceraldehyde 3-phosphate dehydrogenase) were selected according to their expression stability following the various skin treatments.

Upregulation of MRP8 was tested with semiquantitative reverse transcription–PCR on six of 10 volunteers subjected to strong tape stripping (volunteers B, C, E, F, H, and J). In each case, after 25 PCR cycles, no PCR product was detected in control epidermis, whereas PCR products were clearly detected in strongly stripped epidermis (**Fig 5*a***). The band intensities quantification and normalization using ribosomal protein S9 showed that bands detected at 25 cycles after strong tape stripping were 17-fold more intense on average compared with those detected in control epidermis (**Fig 5*b***). For the other skin treatments (moderate tape stripping, vaseline, SDS, SSR), MRP8 modulation was verified in either one or two volunteers (**Fig 5*a***). We showed 2- to 39-fold more intense bands after skin treatment compared with control epidermis (**Fig 5*b***). The upregulation of MRP14 and FRA1 genes following different skin treatments was also confirmed (**Fig 5**). Note that for SDS treatment we chose the volunteers showing the greatest level of induction according to macroarray hybridization experiments.

In conclusion, the results of reverse transcription–PCR confirmed the differential gene expression identified by microarray hybridization for MRP14, MRP8, and FRA1.

Localization of MRP8 and MRP14 in human skin after tape stripping and SSR treatment Histologic analysis of normal human skin after both aggressions revealed morphologic modifications of epidermis. The tape stripping treatment (40 strippings) that immediately removed all the corneocytes (not shown) led 24 h later, to abnormal features of the epidermis with a disappearance of granular layers, formation of parakeratotic covering layers, and increase of epidermal thickness, thus confirming previous data (Pinkus, 1951) (**Fig 6*b***). Twenty-four hours after SSR exposure, sunburn cells located within the suprabasal compartment could be identified in human epidermis, with their typical apoptotic feature, i.e., round shape, eosinophilic cytoplasm, and condensed nucleus (**Fig 6*c***) (Bernerd and Asselineau, 1997).

MRP8 and MRP14 proteins were not detected in normal control human skin (**Fig 6*d,g***) in agreement with previous reports (Saintigny *et al*, 1992; Broome *et al*, 2003). Tape stripping and SSR treatments led to induction of both proteins in epidermis (**Fig 6*e,f,h,i***). A similar pattern of expression was observed for both proteins following either treatment. MRP8- and MRP14-positive keratinocytes were mostly located in the upper part of the epidermis, with a more intense staining at the cellular periphery. The latter characteristic has also been described by Broome *et al* (2003).

DISCUSSION

Environmental stresses induce damage to the skin leading to alterations of the expression of genes implicated in different pathways. The molecular events implicated in early phases (before 2 h) following barrier alteration have been well documented (see *Introduction*). We chose to study later (19 h) molecular changes in order to define new targets allowing a faster return to epidermal homeostasis. Furthermore, the different studies reported until now had been performed *in vitro* or *in vivo*, focused on one or two types of stress and on no more than 10 genes at the same time. In order to obtain a broader physiologic view of the effects of different environmental stresses on gene expression in human skin *in vivo*, our study encompassed several important parameters.

First, all of the experiments were performed *in vivo*, using a total of 28 healthy volunteers.

Secondly, several types of mild aggression mimicking physiologic environmental stresses were tested in parallel. The stresses included a mechanical treatment (tape stripping removing all or part of the SC, which corresponds to a well established model for barrier disruption), two chemical treatments (10% SDS to mimic the effect of a strongly detergent soap or daily application of vaseline considered as a "rustic" cosmetic), and a physical treatment (exposure to 1 MED of SSR, to mimic the effect of sunburn). The intensity of injury was quantified using TEWL for mechanical and chemical treatments and using chromametry for SSR exposure.

Thirdly, we used a large-scale approach to study gene expression with macroarray technology, including 588 different cDNA representing genes implicated in various biologic pathways not restricted to cutaneous biology. This allowed us to have a nonoriented screening. This point was important because of the diversity of stresses tested, in order to get a chance to evaluate better the genes involved in skin stress biology. The restricted quantity of starting biologic material imposed by bioethics forced the experiments to be performed with a technique using the most sensible detection (radioactivity) and the study was then limited to 588 genes. We believe that it is reasonable to choose the Atlas Human cDNA expression arrays (588 named genes, Clontech) because it is a good compromise between statistical significance and the variety of genes studied.

Finally, in order to avoid a variation in gene expression due to sampling error, tissue collection, and cell population heterogeneity described by Cole *et al* (2001) in a previous work, we used a dermatome to obtain 0.2 mm thick epidermal samples with minimal dermal contamination. Moreover, difficulties of evaluation linked to interindividual variability of basal gene expression were avoided by taking the contralateral site of treatment as an internal control for each volunteer.

Skin treatment induced interindividual variability in the level of gene expression modulation Cole *et al* (2001) showed that, although variation of gene expression was observed due to methodologic parameters detailed above, 98% of genes expressed in normal skin were similarly expressed whatever the volunteer. We showed here that following skin treatment, corresponding to tape stripping or SSR exposure, a strong interindividual variability in the intensity of gene expression response was observed. This was particularly true for genes showing strong levels of modulation. Following tape stripping, genes with mean ratios over 15 showed variation coefficients

Table II. Identity and accession number of genes modulated in human epidermis after tape stripping

		Ratio of gene expression											
Accession no.	Gene name	A	B	C	D	E	F	G	H	I	J	Mean	p
Extracellular signaling and communication													
X06233	MRP14	823.9	**443.4**	348.7	1426.2	255.2	920.7	371.5	657.3	65.0	42.3	535.4	<0.001
		195.7	*21.5*	*145.8*	*38.3*	*24.8*	*64.7*	***68.1***	*86.2*	*80.3*	*19.6*	*74.5*	*<0.001*
X06234	MRP8	953.5	**127.3**	**275.3**	777.9	**173.2**	**433.0**	232.0	**336.3**	53.6	**23.8**	338.6	<0.001
		273.5	*3.3*	*36.4*	*10.3*	*20.6*	*20.9*	*32.9*	***38.9***	*28.4*	*9.8*	*47.5*	*<0.001*
M27544	IGF1A	125.3	7.0	15.5	17.1	10.6	28.2	21.8	6.0	7.2	12.6	25.1	<0.001
		39.6	*1.0*	*4.1*	*0.9*	*2.2*	*2.2*	*4.8*	*1.5*	*4.1*	*2.6*	*6.3*	*0.011*
M31159	IGF-binding	*1.3*	*4.1*	*4.1*	*3.3*	*3.0*	*11.5*	*2.0*	*8.7*	*0.9*	*1.1*	*4.0*	*0.028*
	protein 3	1.6	2.2	5.6	5.1	3.6	3.3	2.1		2.4	0.9	3.0	0.010
M60278	Heparin-binding epidermal growth factor-like growth factor	A	23.0	11.3	66.5	2.6	3.5	up	5.0	2.7	A	16.4	0.004
D16431	Hepatoma-derived growth factor	8.2	2.0	2.3	2.3	1.3	2.6	2.2	2.5	1.4	1.9	2.7	<0.001
K03515	Neuroleukin (NLK)	A	1.8	3.3	1.6	5.1	2.1	2.1	1.9	1.4	2.3	2.4	<0.001
M22488	Bone morphogenetic protein 1 (BMP1)	3.4	1.9	1.7	2.0	1.3	2.7	2.2	4.8	2.2	1.3	2.3	<0.001
M92381	Thymosin β-10	5.3	1.4	3.7	4.2	1.9	3.7	2.9	3.5	2.3	2.5	3.2	<0.001
M24545	Monocyte chemotactic and activating factor (MCAF)	*0.3*	*0.4*	*0.3*	*0.2*	*0.5*	*0.6*	*0.1*	*0.7*	*0.8*	*0.3*	*0.4*	*0.008*
X02851	IL-1α	236.8	22.5	39.1	48.8	24.7	89.9	65.8	37.1	77.9	23.3	66.6	<0.001
		79.8	1.6	12.0	2.2	2.8	5.3	11.2	*A*	10.8	*A*	15.7	0.003
M65290	IL-12β	40.8	11.4	26.3	20.8	13.9	31.8	27.9	1.4	11.1	14.0	19.9	<0.001
Cell receptors													
M68932	IL-8 receptor A	A	6.0	2.8	318.3	A	5.6	14.1	2.6	A	A	58.2	0.081
M15169	β_2-adrenergic receptor	0.4	0.4	0.4	0.5	0.4	0.5	0.5	0.7	0.8	0.3	0.5	<0.001
M62424	Thrombin receptor (TR); F2R; PAR1	0.2	0.4	0.3	0.2	0.4	0.3	0.2	0.3	0.8	0.5	0.4	<0.001
Cell surface adhesion													
J04145	Integrin αM	13.4	A	2.1	8.2	3.5	8.6	1.2	A	A	99.8	19.5	0.012
X53586	integrin α6 precursor	1.2	2.7	2.6	4.5	2.3	3.2	3.0	4.1	1.5	2.2	2.7	<0.001
Detoxification/anti-oxidation													
X08058	Glutathione S-	5.9	2.3	3.5	2.6	1.7	5.9	3.4	2.8	4.2	2.5	3.5	<0.001
	transferase pi	*5.4*	*0.9*	*3.1*	*1.2*	*2.2*	*3.0*	*3.1*	*2.3*	*2.7*	*2.4*	*2.6*	*<0.001*

P09488	Glutathione S- transferase mu1	2.2	1.5	2.4	1.7	2.2	2.8	3.2	1.3	1.8	2.0	2.1	<0.001
P00390	Glutathione reductase	3.6	1.9	2.7	1.5	2.7	2.5	4.7	2.7	1.9	2.8	2.7	<0.001
X67951	Thioredoxin peroxidase 2	2.2	1.5	2.7	1.9	2.7	2.5	1.9	2.7	6.7	3.8	2.9	<0.001
S90469	Cytochrome P450 reductase	A	2.1	1.7	2.4	1.3	2.4	3.3	2.4	1.7	1.6	2.1	<0.001
X76648	Glutaredoxin	0.4	0.8	0.3	0.1	0.3	0.3	0.1	0.3	0.9	0.2	0.4	<0.001
Intracellular signaling													
X80692	ERK3	11.0	1.3	2.1	2.0	2.0	2.5	3.0	0.7	1.5	3.7	3.0	0.006
U02082	Guanine nucleotide regulatory protein tim1	0.2	0.7	0.4	0.6	0.5	0.4	0.6	0.5	0.6	0.6	0.5	<0.001
Cell cycle													
D13639	G_1/S-specific cyclin D2 (CCND2)	1.3	3.1	4.1	4.3	2.3	3.8	5.2	2.9	1.3	1.6	3.0	<0.001
U18422	E2F dimerization partner 2	0.3	0.2	0.5	0.3	0.3	0.4	0.2	0.3	0.9	0.3	0.4	<0.001
X55504	Proliferating cell nucleolar antigen P120	0.9	3.3	3.2	4.9	1.0	3.9	5.5	1.9	2.3	1.0	2.8	0.004
Transcription													
X16707	FRA1	15.0	30.0	**74.9**	184.7	12.7	43.8	173.6	28.5	6.0	A	63.2	<0.001
		A	*A*	*A*	*5.4*	***9.0***	*2.9*	*10.5*	*A*	*A*	*3.2*	*6.2*	*0.003*
M83234	YB-1	2.0	2.1	2.8	2.2	2.7	2.4	2.6	3.3	1.7	3.0	2.5	<0.001
L34587	Transcription elongation factor B (SIII)	5.1	3.4	2.3	1.9	1.5	2.2	2.2	2.1	2.2	1.6	2.4	<0.001
M19720	L-myc	0.4	0.7	0.4	0.1	0.5	0.4	0.1	0.4	1.2	0.8	0.5	0.006
Apoptosis													
Y09392	Death domain receptor 3 (DDR3)	3.5	1.7	3.4	1.4	3.2	2.9	3.1	1.3	2.0	1.6	2.4	<0.001
Stress response													
X07270	Heat shock 90 kDa protein A	2.1	1.8	1.3	2.5	2.1	3.5	3.3	2.2	2.3	2.1	2.3	<0.001
DNA repair													
U07418	DNA	4.7	3.4	2.9	2.6	2.8	5.0	3.4	2.1	3.1	2.3	3.2	<0.001
	mismatch repair protein MLH1	*4.5*	*1.5*	*3.2*	*1.9*	*2.5*	*3.2*	*3.0*	*1.4*	*2.5*	*2.7*	*2.6*	*<0.001*
Other													
	Cytoplasmic β-actin	1.0	6.3	2.7	3.2	2.6	3.1	2.4	3.2	1.5	1.7	2.8	<0.001
	GAPDH	1.4	2.0	3.8	2.2	2.7	3.5	2.7	4.1	1.2	2.1	2.6	<0.001
	Brain-specific tubulin α1 subunit	1.8	1.7	1.8	2.6	2.4	2.3	2.4	5.4	1.4	2.6	2.5	<0.001

The ratio of gene expression was determined by the normalized expression level in control epidermis divided by the normalized expression level in treated epidermis of a same subject. p-values for Student's t test are indicated in the last column (comparison of geometric mean ratio with the 1-value). Modulations confirmed by semiquantitative reverse transcription–PCR are in bold. When expression in control and treated epidermis was below baseline, the ratio of gene expression was marked as absent (A). When expression in control sample was below baseline, the ratio of gene expression could not be calculated (division by 0) and was marked as upregulated (up). ↑, upregulation; ↓, downregulation.

Table III. Identity and accession number of genes modulated in human epidermis after daily application of Vaseline

		Ratio of gene expression							
Accession no.	Gene name	K	L	M	N	O	P	Mean	p
Extracellular signaling and communication									
X06233	MRP14	5.3	4.6	**3.2**	2.5	4.2	1.4	3.5	0.002
X06234	MRP8	4.2	2.2	**3.1**	3.4	3.2	0.9	2.8	0.01

The ratio of gene expression was determined by the normalized expression level in control epidermis divided by the normalized expression level in treated epidermis of a same subject. p-values for Student's t test are indicated in the last column (comparison of geometric mean ratio with the 1-value). Modulations confirmed by semiquantitative reverse transcription–PCR are in bold.

Table IV. Identity and accession number of genes modulated in human epidermis after SSR exposure

		Ratio of gene expression							
Accession no.	Gene name	Q	R	S	T	U	V	Mean	p
Extracellular signaling and communication									
X06233	MRP14	16.9	193.8	1.1	**32.4**	3.5	3.5	41.9	0.03
X06234	MRP8	2.8	53.3	0.9	**12.4**	2.1	2.1	12.3	0.06
X78686	Granulocyte chemotactic protein 2	8.2	6.4	A	7.7	A	A	7.4	0.001
Cell cycle									
M60974	GADD45	1.7	3.9	1.0	5.2	1.6	2.2	2.6	0.02
Transcription									
X16707	FRA1	4.0	20.1	0.5	up	**8.3**	16.8	10.0	0.06
Apoptosis									
L22474	Apoptosis regulator bax	2.5	3.4	8.2	3.4	1.8	1.8	3.5	0.005
Other									
U01160	SAS (transmembrane 4 superfamily protein)	3.2	2.9	A	A	2.1	1.0	2.3	0.06

The ratio of gene expression was determined by the normalized expression level in control epidermis divided by the normalized expression level in treated epidermis of a same subject. p-values for Student's t test are indicated in the last column (comparison of geometric mean ratio with the 1-value). Modulations confirmed by semiquantitative reverse transcription–PCR are in bold. When expression in control and treated epidermis was below baseline, the ratio of gene expression was marked as absent (A). When expression in control sample was below baseline, the ratio of gene expression could not be calculated (division by 0) and was marked as upregulated (up).

Table V. Identity and accession number of the common genes modulated in different conditions of treatment

		Mean ratio of gene expression			
		Tape stripping			
Accession no.	Gene name	Strong	Moderate	SSR	Vaseline
Extracellular signaling and communication					
X06233	MRP14	535	74	42	4
X06234	MRP8	339	47	12	3
Transcription					
X16707	FRA1	63	6	10	

Significant mean ratio of gene expression is indicated for each treatment.

ranging from 80 to 220% (see, for example, in **Table I** MRP14, MRP8, IL-1α or FRA1). Following SSR exposure, the most upregulated genes FRA1, MRP8, and MRP14 showed the greatest interindividual variability.

Moreover some volunteers were globally more responsive than others, although all volunteers received the same treatment intensity (see volunteer A in **Table II** and volunteer R in **Table III**). This illustrates the well-known heterogeneity of *in vivo* individual response to stress. Although the volunteers were included in protocols with defined criteria of age, phototype, and skin history, leading to a homogeneous cohort, other important parameters may participate to influence gene response following skin treatment.

Nature and intensity of skin treatment influenced the number of modulated genes This study showed an absence of response, or a response with low significance, 19 h after acute application of 10% SDS or after daily application of vaseline. Indeed, barrier function was not altered under both experimental conditions as measured by TEWL and the number of genes found modulated was very low (none of 588 and two of 588 for SDS and vaseline treatment, respectively). This absence of long-lasting significant response underlines the innocuousness of these compounds frequently found in cosmetic products.

In contrast, the harsh mechanical stress exhibited a strong long-lasting response of gene expression correlated with an increased TEWL compared with control skin. Indeed, following tape stripping, increased barrier disruption correlated with an increased number of modulated genes and with increased intensity of gene response, indicating a tape stripping dose-effect.

Different skin treatments led to different and common gene modulations.

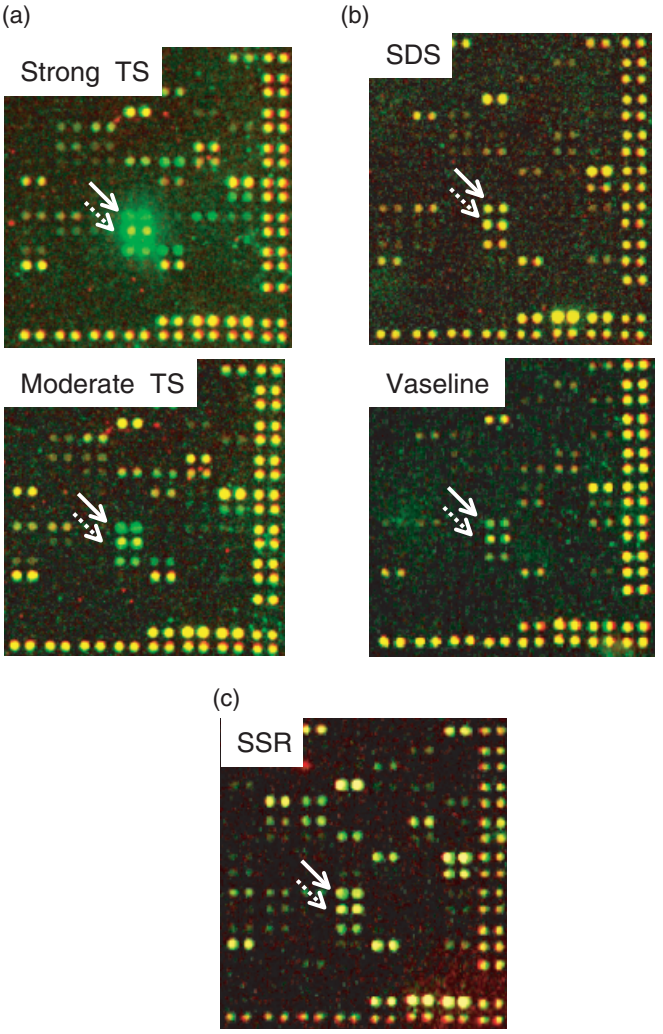

Figure 4. Portions of Atlas Human cDNA expression array representative of differential hybridizations. Differential hybridization of Atlas Human cDNA expression array after mechanical stress (*a*: strong or moderate tape stripping on volunteer F), after chemical treatment (*b*: 10% SDS on volunteer W or vaseline on volunteer O), and after SSR (*c*: 1 MED of SSR on volunteer Q). Using Imagene Software, the filters hybridized with ^{33}P-labeled cDNA probes derived from control and treated epidermis of the same volunteer were overlaid: the treated sample was colored in green; the control sample was colored in red. Genes that were upregulated, downregulated, or not modulated appeared green, red, or yellow-colored, respectively. *Full and dashed arrows* indicate signals for the MRP14 and MRP8 genes, respectively.

Genes modulated by SSR exposure only The modulation of four genes was specific to the mild and physiologic SSR exposure as neither tape stripping, nor vaseline, nor SDS application altered their expression. They include GADD45, Bax, SAS, and granulocyte chemotactic protein-2.

GADD45 (growth arrest and DNA damage-inducible gene 45) is associated with cell growth inhibition, DNA damage response, increased DNA repair, and inhibition of cell death in keratinocytes (Maeda *et al*, 2002). It is induced by base damaging agents, such as methylsulfonate, UV radiation, and other ionizing radiation (Hollander *et al*, 1993). Its activation has been shown to be p53 dependent (Zhan *et al*, 1994).

Bax is implicated in normal keratinocyte differentiation and response to genotoxic stress *in vivo* and may provide a tumor suppressor effect during skin carcinogenesis (Cho *et al*, 2001). GADD45 and Bax, have already been shown to be induced following UV exposure of human skin (O'Grady *et al*, 1998; Wan *et al*, 2000). These results underscore the reliability of the gene screening procedure used. Furthermore, we showed that GADD45 and Bax genes could be induced in human skin by SSR, which corresponds to a combination of UVB + UVA representing the more physiologic UV source. Although UVB wavelengths represent only a small percentage in the SSR source, as in solar radiation, its high energetic properties and its direct DNA-damaging effects were probably responsible for these gene modulations.

To our knowledge SAS and granulocyte chemotactic protein-2 have never been shown to be implicated in UV stress response.

SAS (sarcoma amplified sequence) belongs to the recently described gene family "transmembrane four" (TM4) that encodes a group of cell surface proteins all possessing four conserved transmembrane domains. SAS is amplified in surface osteosarcoma and is thought to be involved in growth-related cellular processes (Jankowski *et al*, 1994).

Granulocyte chemotactic protein-2, also known as epithelial neutrophil-activating protein 78 (ENA78), is a member of the Cys-X-Cys chemokines and acts as a potent chemoattractant and as an activator of neutrophil function. The level of granulocyte chemotactic protein-2 is strongly elevated in the process of inflammatory diseases (e.g., psoriasis, rheumatoid arthritis, Crohn's disease, ulcerative colitis, and acute appendicitis) (Chang *et al*, 1994; Walz *et al*, 1997). Granulocyte chemotactic protein-2 could play a part in UV-induced inflammation of human epidermis.

Genes modulated by tape stripping only Tape stripping leads to barrier disruption, produces cellular damage due to water loss of the surface layers, and induces hyperemia, a mild inflammation in the corium, and epidermal hypertrophy. All of these factors are well recognized as associated with epidermal proliferation (Pinkus, 1951).

Nineteen hours following strong tape stripping, the inflammation phase was still present with the overexpression of pro-inflammatory cytokines IL-1α and IL-12. Other *in vivo* studies showed the early induction (as early as 1 h following tape stripping) of IL-1α and various other cytokines (Wood *et al*, 1992, 1997).

Furthermore, genes coding for proteins playing a part in cellular detoxification and anti-oxidation were modulated (glutathione S-transferase mu1, glutathione reductase, thioredoxin peroxidase, cytochrome P450 reductase, and glutaredoxin), suggesting that tape stripping induced free radicals of endogenous origin. They are most likely produced by infiltrating neutrophils and macrophages that generate and release an array of reactive oxygen species into the extracellular milieu (Athar *et al*, 1992). These results support the hypothesis that oxidative stress plays a part in tape stripping-induced inflammation.

Several genes involved in cell proliferation were overexpressed, such as IGF1A, and IGFBP3 (Phillips *et al*, 1998), P120 antigen (Valdez *et al*, 1992), thymosin β 10 (Santelli *et al*, 1999), or cyclin D2 (Zhang *et al*, 1997), whereas some genes implicated in cellular differentiation were repressed, such as thrombin receptor (PAR1) or L-myc (Morgenbesser *et al*, 1995). These modulations probably reflected the proliferative response of epidermal keratinocytes and could be linked to a healing process aimed at re-establishing the disrupted SC.

It is important to note that due to differences in signal intensity between samples from the different skin treatments, it is difficult to firmly establish whether the genes only modulated by tape stripping are really specific for this treatment. Indeed, it may be possible that following SDS application a similar gene response would be observed at an earlier time point, or at the same time with a higher SDS concentration.

Genes modulated by different epidermal stresses FRA1 was induced after tape stripping and SSR exposure. This gene is a member of the Fos gene family, which encodes proteins that are

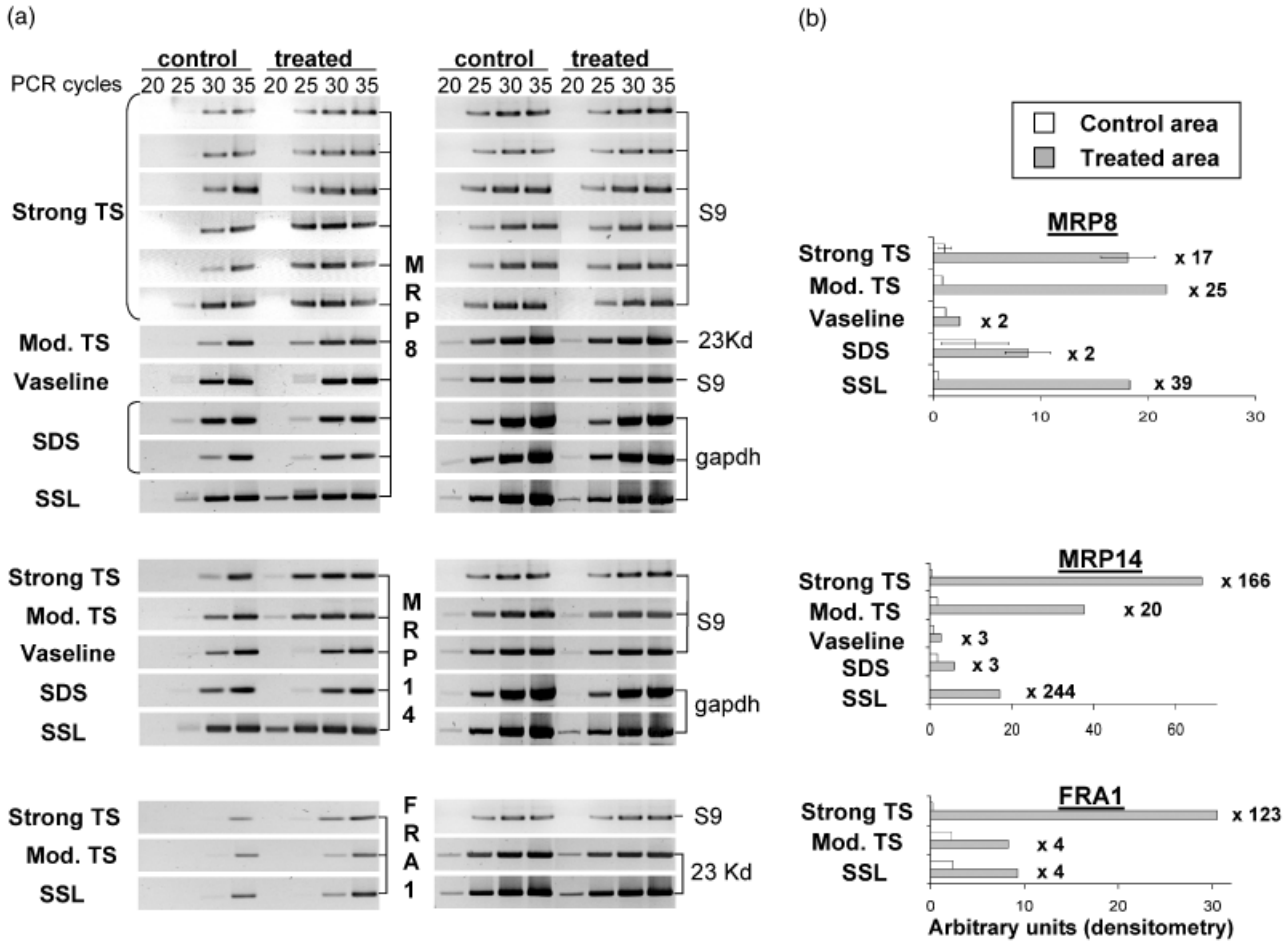

Figure 5. Confirmation by semiquantitative reverse transcription–PCR of the cDNA microarray-identified differential expression of selected genes after different treatment conditions of human epidermis. (*a*) Reverse transcription–PCR. *Lanes 1–4*, control epidermis; *lanes 5–8*, treated epidermis. *Left panel*: genes found modulated. *Right panel*: housekeeping genes, S9, ribosomal protein S9; 23 kDa, 23 kDa highly basic protein; GAPDH, liver glyceraldehyde 3-phosphate dehydrogenase. (*b*) Quantification of reverse transcription–PCR. For MRP8 and MRP14, bands at 25 cycles were quantified (except for SSR, 20 cycles). For FRA1, bands at 25 cycles were quantified. Normalization was done with bands at 25 cycles (except for SSR, 20 cycles) of housekeeping gene. Ratio control/treated area of band intensity is given beside the histogram bar.

part of the activator protein-1 (AP-1) transcription factor complex. The AP-1 pathway is implicated in skin differentiation (Fisher *et al*, 1991; Dlugosz and Yuspa, 1993; Basset-Seguin *et al*, 1994) and in the regulation of several epidermal genes (Yamanishi *et al*, 1992; Bernerd *et al*, 1993; Lu *et al*, 1994). It is also well known that UV exposure results in upregulation of several oncogenes, including members of the Fos family. In addition, UV is responsible for AP-1 induction leading to the modulation of several genes containing AP-1 regulatory sequences, as illustrated by matrix metalloproteinases (Wisdom, 1999).

MRP8 (also known as calgranulin A or S100A8) and MRP14 (also known as calgranulin B or S100A9) were both found to be upregulated following tape stripping, vaseline, and SSR treatments and showed the highest rates of modulation. These proteins belong to the S100 protein family. S100 proteins are intracellular Ca^{2+}-binding and intracellular Ca^{2+}-modulated proteins that form anti-parallel non covalently linked dimers in solution and play a part in various Ca^{2+}-mediated cellular and extracellular functions (Donato, 1999). MRP8 and MRP14 are expressed constitutively in neutrophils and monocytes and, after activation, in macrophages, they are secreted by these cells (Kerkhoff *et al*, 1998). Proinflammatory activities of both proteins were recently detailed by Ryckman *et al* (2003) who showed that MRP8 and MRP14 induced neutrophil chemotaxis and adhesion, and suggested their role in neutrophil stimulation and migration to inflammatory sites. In normal skin, MRP8 and MRP14 are not detected in the interfollicular epidermis. Their expression is restricted to the infundibulum of hair follicles (Brandtzaeg *et al*, 1987). Under certain physiologic or pathologic conditions, however, they can be both upregulated, especially in inflammatory and/or hyperproliferative conditions. For example, MRP8 and MRP14 are upregulated in psoriatic epidermis (Madsen *et al*, 1992; Saintigny *et al*, 1992), in epithelial skin tumors where they have been described as tumor-associated genes (Gebhardt *et al*, 2002), during the hyperproliferative phase of wound healing (Thorey *et al*, 2001), and in cultured human keratinocytes (Saintigny *et al*, 1992). In involved psoriatic skin, both proteins are localized at the plasma membrane of keratinocytes of the basal, spinous, and granular layers (Broome *et al*, 2003). During wound healing, they were found in differentiating suprabasal keratinocytes (Thorey *et al*, 2001).

This study shows a common induction of MRP14 and MRP8 mRNA in normal human epidermis following mechanical, chemical, and SSR treatments. Such upregulation was confirmed at the protein level after tape stripping or SSR exposure. Our results also confirmed the absence of MRP8 and MRP14 proteins in normal human skin as well as their high pattern of distribution at the periphery of the keratinocytes after skin injuries. Following tape stripping, a model for epidermal regeneration, a concentration-dependent response can be observed for those genes, suggesting that barrier disruption modulates their expression. If one considers that the upper follicle is the

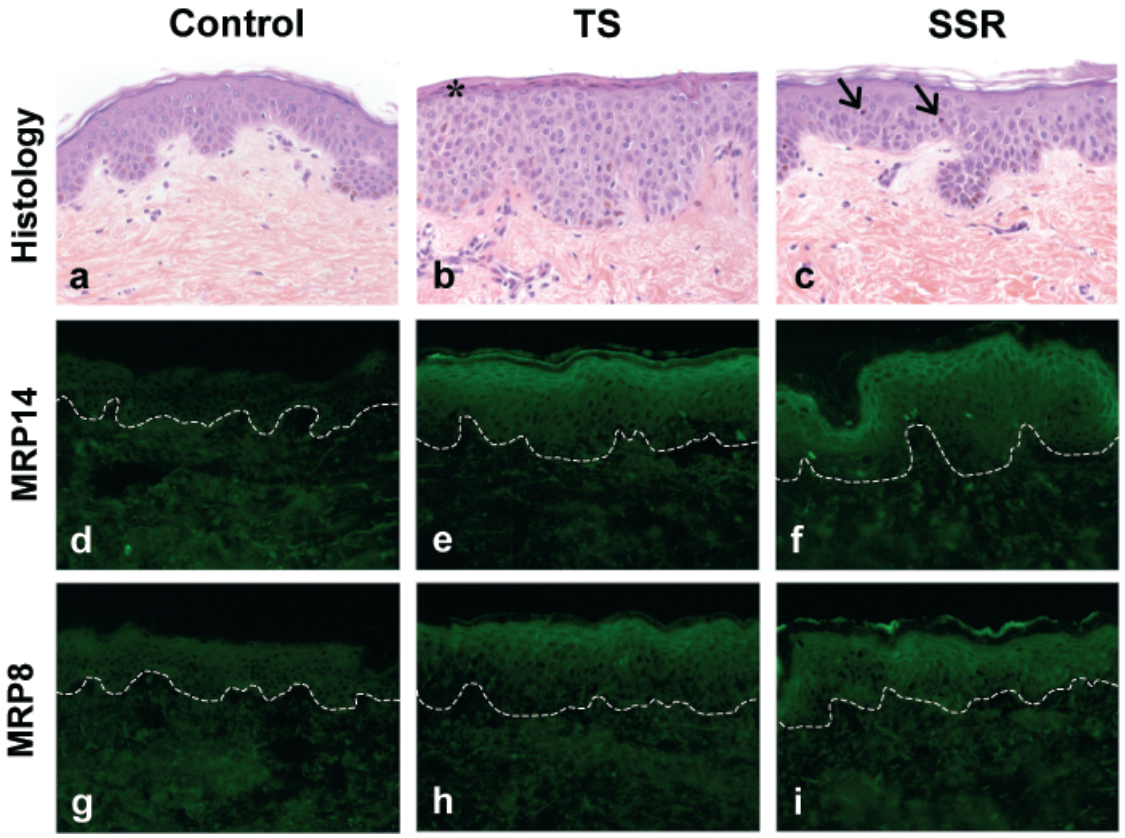

Figure 6. Histology and MRP14 and MRP8 immunolabelings in human skin following tape stripping or SSR exposure. Classical histology: (*a–c*) MRP14 detection; (*d–f*) MRP8 detection; (*g–i*) normal human skin was analyzed 24 h after treatment: (*a,d,g*) control condition; (*b,e,h*) 40 tape strippings; or (*c,f,i*) SSR exposure. Basement membrane zone is shown with white dotted lines. *Arrows* indicated sunburn cells. Star indicated parakeratotic covering layer.

keratinocyte reservoir for emergency wound closure (Lenoir *et al*, 1988; Taylor *et al*, 2000), MRP8 and MRP14 expression might be a prerequisite for active epidermal renewal and keratinocyte hyperproliferation. Moreover their potential anti-microbial activity (Brandtzaeg *et al*, 1995) might prevent superinfection under these circumstances where barrier function and SC integrity are altered. MRP8 and MRP14 expression might thus represent a general marker of epidermal regeneration. SC integrity is not the only factor associated with MRP8 and MRP14 upregulation, however, as both genes were found overexpressed following SSR exposure and vaseline treatment. Indeed, following these treatments no alteration or protection of the cutaneous barrier could be observed (Haratake *et al*, 1997) (**Fig 3**). In the case of SSR, the induction of MRP8 and MRP14 could be associated with epidermal proliferation subsequent to cell growth arrest. Altogether, our results suggest that MRP14 and MRP8 are general markers for skin response to a great variety of stresses, and that their implication is not restricted to epidermal regeneration and inflammation.

CONCLUSIONS

This study demonstrates the value of cDNA microarray gene profiling to characterize genes modulated in response to a given stimulus and to compare different cutaneous treatments. Part of our data is consistent with those previously obtained by other technical means thus giving a validation of the approach. In addition, new results in terms of modulated genes were obtained and a global comparative analysis between treatments was performed. These data and probably future experiments using high-density and/or tissue-specific microarrays will be helpful to identify pertinent target genes for the prevention or treatment of skin disorders.

The authors thank F. Christiaens for monitoring the UV source, D. Asselineau, C. Gerst, and D. Bernard for helpful discussion, Dr S. del Bino-Nokin and C. Vioux are acknowledged for their help in protein experiments.

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